Theoretical Analysis of Optogenetic Mediated Neural Spiking

A synopsis of the proposed thesis to be submitted for the degree of

DOCTOR OF PHILOSOPHY

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1. Introduction

The human brain consists of more than 100 billion neurons forming a dense network through 100 trillion synapses. Within brain circuits, information about sensory stimuli is encoded in complex spatial and temporal patterns of activity distributed across the cells. To causally test which sensory features are encoded in neural circuits and how this information is used to drive behaviour, it is necessary to read out the behaviour of individual subcomponents with high precision, as well as to modulate their activity.

Optogenetics, a new technique was introduced into the field of neuroscience in 2005 (Boyden et al., 2005). Optogenetics refers to the integration of genetic targeting strategies of light-sensitive proteins to achieve gain or loss of function of precisely defined events within specified cells, tissues and living animals with optical control (Kim et al., 2017; Deisseroth et al., 2006; 2011). This technology has revolutionized the field of neuroscience and cell biology by enabling unprecedented spatial and temporal resolution with minimal invasiveness. Optogenetics has found widespread applications not only in neuroscience (Zhang et al., 2006; Han and Boyden et al., 2007; Yizhar et al., 2011a), but also in the field of cardiology (Williams et al., 2013; Bruegmann et al., 2015), gene transcription of cell (Olson et al., 2017) and cell signaling (Repina et al., 2017).

In the last decade, the evolving field of optogenetics has provided opportunities for numerous applications that include cortical circuit mapping (Wang et al., 2007; Zhang et al., 2007; Klapoetke et al., 2014), control of motor cortex (Aravanis et al., 2007), regulation of the Parkinson's symptoms of neurodegenerative disorder (Gradinaru et al., 2009) and closed loop control of epileptic seizures (Paz et al., 2015; Tonnesen et al., 2017).

The development of optogenetics has followed two parallel paths, one focusing on the development of light-sensitive proteins, called opsins, for the genetic modification of neurons and the development of optoelectronic and photonic technologies for light delivery.

To date a wide variety of naturally occurring and synthetic microbial rhodopsins have been engineered to enable optical excitation, inhibition, bistable modulation and control of biochemical

signaling in neurons (Zhang et al., 2011; Govorunova et al., 2017a). In short, neurons are first genetically engineered to express light-sensitive proteins called opsins. When these neurons are illuminated with light of correct frequency, they can be transiently activated or inhibited or their signaling pathways can be modulated, depending on the particular kind of opsin that was chosen for expression. Light can be applied in a variety of temporal patterns in order to optimally influence neuronal function (Guru et al., 2015). In optogenetics, microbial rhodopsin pumps and channels are the most widely used opsins (Govorunova et al., 2017a). The millisecond kinetics of the microbial rhodopsins permits neuronal modulation with precise onset and offset (Deisseroth et al., 2006).

The most studied member of the microbial rhodopsin family called Channelrhodopsin-2 (ChR2), isolated from the fresh water algae *Chlamydomonas rheinhardtii*, was the first opsin to control the spiking activity of neurons with millisecond temporal precision (Boyden et al., 2005). ChR2 is a light-gated nonspecific cation channel, which in response to blue-light, opens and allows the passage of cations and the subsequent depolarization of the cell (Nagel et al., 2003; 2005). This initial demonstration with ChR2 was soon followed by a number of reports confirming its function in various types of neurons (Li et al., 2005; Ishizuka et al., 2006).

After successful applications of ChR2, it was engineered to optimize expression in mammalian systems (Nagel et al., 2005; Gradinaru et al., 2007). Through these efforts, opsins with ultrafast kinetics and improved photocurrent, such as ChETA and ChEF/ChIEF were developed, among others (Lin et al., 2009; Gunaydin et al., 2010; Mattis et al., 2012). Due to the ability of red light to penetrate deeper into the tissue, various opsins that include VChR1, C1V1, ReaChR, Chronos, Chrimson, vf-Chrimson, have been genetically engineered (Zhang et al., 2008; Lin et al., 2013; Klapoetke 2014; Mager et al., 2018).

To modify the neural population's spontaneous firing rate rather than spike generation control, step-function opsins (SFOs) such as ChR2 (C128A), ChR2 (C128T) and ChR2 (C128S) have been developed by altering ChR2 with excitation wavelength (ON by 470 nm and OFF by 546 nm), with a turn-on time of 7.2 ± 0.8, 11.6 ± 1.5 and 20 ± 1.4 ms, and turn-off time of 52 ± 2, 2 ± 0.5 and 106 ± 9

s, respectively (Berndt et al., 2009). Further efforts have led to stable SFO-ChR2 (C128S/D156A) with turn-on of 10 ms and turn-off of 29 min, which has nearly half an hour of spontaneous deactivation life (Yizhar et al., 2011b). Using these opsins, the photocurrent can be launched with a short blue light pulse and inhibited with a yellow light pulse. Another benefit is that these opsins are highly sensitive to light which makes them beneficial for use in a light source several millimeters away in neurons (Tye and Deisseroth, 2012).

Further, the SFO kinetic principle is applicable to inhibitory ChRs, creating bistable and extremely light-sensitive inhibitory ChRs that include SwiChR (turn off of 7.3s) and SwiChR++ (turn off of 150 ± 20ms), with activation wavelength (on by 475 nm and off by 600 nm) (Berndt et al., 2014, 2015). SwiChR++ enabled temporally delimited bistable control over chloride conductance and neuronal inhibition and found utility in studies of mouse behaviour (Iyer et al., 2016).

The first reported optogenetics inhibition used NpHR, a halorhodopsin from *Natronomonas pharaonis* (Zhang et al., 2007; Han and Boyden, 2007). In response to 593 nm light, NpHR causes hyperpolarization of neurons or inhibition of spiking by pumping chloride ions into the cell with turn-on and turn-off time of the order of ms (Zhang et al., 2007; Han and Boyden, 2007). Further experiments used genome screening, rationally design mutagenesis and molecular trafficking strategies to discover and engineer new inhibitory opsins with improved kinetics and photocurrent, that includes eNpHR, eNpHR2.0, eNpHR3.0, Arch, ArchT, Mac, eBR (Gradinaru et al., 2008; 2010; Chow et al., 2010; Han et al., 2011). More recently, a red-shifted cruxhalorhodopsin called Jaws, a chloride pump from *Haloarcula salinarum*, has been discovered and optimized to provide higher photocurrent three times that of earlier inhibitory opsins (Chuong et al., 2014).

Recent engineering efforts along with the identification of naturally occurring anion-conducting channelrhodopsins have generated a completely new class of optogenetic inhibitory opsins that includes iC++ and iChloC. These eACRs have shown high selectivity of anions (Berndt et al., 2015; Wietek et al., 2015; Kato et al., 2012). Wietek et al. demonstrated chloride-conducting ChRs (ChloCs) having excitation wavelength of 470 nm (Wietek et al., 2014).

New screening attempts have recently revealed a new class of natural anion channelrhodopsins (nACRs) from various cryptophyte species that deliver extremely delicate and effective hyperpolarization and neuronal silencing through selective anion conduction (Govorunova et al., 2015). The nACRs obtained from *Guillardia theta*, called GtACR1 & GtACR2, demonstrated full inhibition of electrical cardiomyocyte activity under circumstances, where Arch was inefficient (Govorunova et al., 2016). Another remarkable version named ZipACR showed a large variety in amplitude, kinetics (present half-decay time 2-4 ms) and spectral sensitivity (with excitation wavelength of 520 nm) of its photocurrents. In cultured mouse hippocampal neurons, ZipACR allows accurate photoinhibition of individual spikes from trains up to 50 Hz (Govorunova et al., 2017b).

Due to the high absorption and scattering coefficients, visible light cannot reach the inner depths of the brain (Chuong et al., 2014). On the other hand, infra-red light sources, used in two-photon excitation technique, provide better spatial specificity and deeper penetration in the tissue. Two-photon optogenetic neuronal activation, providing accurate targeting of neurons and reduced scattering in tissue, has been realized by employing of two approaches that includes the scanning and the parallel method (Helmchen and Denk, 2005; Packer et al., 2012; 2015; Yang et al., 2018).

Two-photon optogenetic activation of neurons was originally demonstrated with ChR2 (Rickgauer and Tank, 2009). Consequently, it has also been observed with red-light activated variants eg. C1V1, ReaChR (Packer et al., 2012; 2015; Prakash et al., 2012; Chaigneau et al., 2015). Furthermore, two-photon holographic photoactivation of Chronos, ChroME, and CoChR has been reported allowing sub-millisecond optogenetic control of neuronal firing (Ronzitti et al., 2016; Mardinly et al., 2018; Shemesh et al., 2017). More recently, two-photon holographic illumination of anion conducting channelrhodopsin GtACR2 (with excitation wavelength 920 nm; stimulus power 30 mW) has been shown, which efficiently decreased neuronal firing with high spatial resolution, *in vivo* (Forli et al., 2018).

Recently, the control of the cardiac tissue's excitable properties augmentation or suppression using optogenetic actuators (microbial opsins), have also been demonstrated. In the genetically modified

myocytes expressed with ChR2, blue light illumination generates robust depolarizing transmembrane current leading to the development of action potentials. Further, ChR2(H134R)-expressing mouse embryonic stem cells derived cardiomyocytes have been reported which contract in response to short blue-light flashes and accelerate their spontaneous beating rate following extended blue-light activation (Bruegmann et al., 2015). Also, bi-directional light-based control of cardiomyocyte membrane potential (*i.e.*, both depolarization and hyperpolarization) has been shown by co-expressing the excitatory and inhibitory opsins (Nussinovitch et al., 2014).

Recent efforts have led to characterization of both light and voltage-sensitive properties of ChR2 photocurrent and its operation in cardiomyocytes (Williams et al., 2013). The inclusion of an empirical scaling function to represent the voltage rectification of ChR2 was found to be essential in accurate reproduction of ChR2 photocurrent behavior during various phases (upstroke, notch, plateau, repolarization) of light-triggered cardiac action potentials. Suppression of spontaneous beating of monolayers expressing light-gated anion conducting channelrhodopsin (GtACR1), in response to blue light pulses, have been shown. Most importantly, ACR expression enabled accurately controlled shortening of the possible duration of the action by switching on the light during its repolarization phase which was not possible with previously used optogenetic instruments (Govorunova et al., 2016).

2. Computational Optogenetics

Computational optogenetics has offered tremendous opportunities to not only develop better understanding of optogenetic experiments but also in exploring its applications from single neuron to network level (Grossman et al., 2011). Due to the naturally occurring structural diversity in living animals and tissues, a huge amount of data-set is required to be generated for optogenetics for its use under different circumstances, which is a challenging task. The task becomes more challenging while considering the diversity in the photocycle and functioning of opsins used in optogenetics, more than hundred in numbers. In that case, computational optogenetics assists in providing pre-defined and highly precise values of parameters such as intensity, wavelength, pulse-width and concentration to

design efficient experiments. Such simulations can also drive the rational design of new and optimized optogenetic experiments for a particular cell/tissue setting. Computational optogenetics aims to formulate accurate model of the photoresponse of light-sensitive proteins and to integrate it with an accurate neuron circuit model to analyse light-evoked response in opsin-expressing neurons.

From the modeling perspective of photoresponse of opsins, three transition rate models, 3-state, 4-state and 6-state models of ChR2 photocycle, are presently available. The models not only accurately capture the photoresponse but also provide a deep understanding of the photocycle of ChR2 (Nikolic et al., 2006; 2009). In order to simulate optogenetically evoked spiking in ChR2-expressing neurons, subject to different stimulation protocols, Grossman et al. formulated an integrated model by combining the photocycle model of ChR2 and neuron circuit model (Grossman et al., 2011). Grossman *et al.*, have also investigated how the spatial pattern of excitation affects the response of ChR2 expressing neurons via simulation (Grossman et al., 2013). Further, the photoresponse of ChETA and ChRET/TC were modelled (Stefanescu et al., 2013). In order to capture the dynamical response of the neurons expressing these variants, the model was incorporated in slow (Hodgkin-Huxley neuron model) and fast (Wang-Buzaski interneuron model) spiking neuron circuit models (Hodgkin and Huxley, 1952; Wang and Buzaski, 1996). A multi-scale computational tool, called PyRhO, has recently been created to model the photocurrent of opsins by automatically fitting a minimum number of experimental parameters into three, four or six-state kinetic models and provide a platform to integrate these models at channel, neuron and network level (Evans et al., 2016).

Recently, an accurate model of the photoresponse of Chronos-expressing neurons has been reported (Saran et al., 2018). Theoretical analysis of low-power, fast optogenetic excitations of red-shifted vf-Chrimson-expressing neurons have also been reported (Gupta et al., 2019).

Optogenetics at present is spearheaded by experimental research. Hence, there is tremendous scope and requirement for computational optogenetics. Computational modeling of cardiac optogenetics incorporates the light- and voltage-sensitive proteins. Photostimulation causes transient flow of

transmembrane ion-currents (Boyle et al., 2013; Jiang et al., 2017). However, the detailed theoretical analysis and optimization remains to be studied.

3. Motivation

Computational optogenetics provides aid in quick (virtual) testing of the photoresponse of newly developed opsins in a wide range of cell types and within realistic tissue settings. Such simulations also provide insights to design new improved opsins and can be optimized for a specific cell/tissue environment. It assists to understand experiments within a complex cell/tissue environment, which are often ambiguous and challenging to interpret (Williams et al., 2013).

In case of long duration excitation of neural circuitry, continuous illumination of light is required, which would heat the tissue. Many physiological processes are temperature-sensitive. Commonly used illumination protocols increased the temperature by 0.2-2^{0}C and suppressed spiking in multiple brain regions (Owen et al., 2019). In those cases, opsins with bi-stable photoresponse are best suited, to generate a stable excitatory or inhibitory photocurrent up to seconds, using a brief pulse of light of an order of several milliseconds.

In situations requiring long-lasting inhibition, the pumps could potentially influence the cellular-ion homeostasis (Weigert et al., 2017). Moreover, one-to-one stoichiometry between photon absorption and ion-pumping necessitates constant illumination to achieve continued hyperpolarization or silencing, setting an upper bound on the volume of the modulated area due to risk of heating and photo-damage (Wiegert et al., 2017). On the other hand, ACRs ideally conduct many ions per absorbed photon and produce several-fold larger photocurrents. Two-photon stimulation of GtACR2 has been demonstrated which have shown larger peak amplitude at 920 nm that decreases at longer and shorter wavelengths (Froli et al., 2018).

Although, accurate modeling of the photoresponse of fast excitatory (ChR2, Chronos, vf-Chrimson) and inhibitory (NpHR) channelrhodopsins and light-controlled neuronal signaling of neurons expressing them have been reported (Nikolic et al., 2013; Saran et al., 2018; Gupta et al., 2019). The

photoresponse of step-function opsins, having applications for low power long-lasting optogenetic excitation and inhibition, remain to be studied. Also a wide variety of ACR–mediated optogenetic inhibitions, having rapidly growing applications and their two-photon excitation need to be theoretically analyzed. So far, to the best of our knowledge, theoretical models of these opsins and opsin-expressing neurons have not been reported as yet.

4. Objectives

For bistable control, step-function opsin (SFO) shows step-like control of neuronal membrane potential that can bring cells closer to action potential threshold and increase the probability of spiking to endogenous synaptic inputs (Berndt et al., 2009). This approach may be particularly useful in situations where more naturalistic and desynchronized spiking patterns are preferred.

These 'bi-stable' channelrhodopsins are based on point mutations at the C128 position. Three different point mutations at the C128 position have been shown to give rise to ChR2 variants with slow channel closing kinetics (Berndt et al., 2009). ChR2(C128T) has a closing time constant of 2 s, whereas ChR2(C128A) and ChR2(C128S) are characterized by even slower channel closure and time constants of 52 s and 106 s, respectively (Berndt et al., 2009). The C128A mutant has very interesting kinetics. Its open state outlasts the activation light pulse by many orders of magnitude, but it still spontaneously inactivates within an experimentally accessible time window. Berndt et al., used the C128A mutant to induce long and reversible subthreshold depolarizations in dissociated neurons and to sensitize cells to synaptic input (Berndt et al., 2009). As it has faster kinetics of activation and superior green light inactivation, the C128A mutant may be best suited for experiments requiring fast activation and inactivation of photocurrents. Schoenenberger et al. have showed the effects of ChR2(C128A) activation when the mutant channel is expressed at high levels in pyramidal cells in hippocampal slice culture (Schoenenberger et al., 2009).

The double-mutant protein is referred as a stable SFO (SSFO). It has been shown that the double mutant C128S/D156A, exhibits notable stability of the enabled state, with little detectable return to the dark state after 30 minutes. It can be deactivated with either 590 or 405 nm light at intensities as

low as 8 mW/mm^2 and generates hundreds of picoamperes of whole-cell photocurrent in neurons expressing SSFO (Yizhar et al., 2011a). Recently, SFOs have been shown to deliver bistable optogenetic control in muscle cells and *C. elegans* neurons and in the brains of awake, behaving primates (Diester et al., 2011; Schultheis et al., 2011). Additional and combinatorial mutagenesis based on these initial principles has led to additional SFOs with time constants of deactivation up to 30 min (Bamann et al., 2010; Yizhar et al., 2011a). Several advantages accrue from application of SFOs to optogenetics: 1) Light need not to be provided continuously. The many-orders-of-magnitude reduction in energy delivered can eliminate any intense-light toxicity from long-term experiments. 2) Continuous mild depolarization can simply favour excitability of targeted cells rather than provide precise user-defined spikes, an approach often leveraged to maintain or enhance native rhythm/timing relationships (Berndt et al., 2009; Mattis et al., 2011; Yizhar et al., 2011; Schmitt et al., 2017; Ferenczi et al., 2016; Iyer et al., 2016).

Moreover, integration of diverse structural insights has started. It was found that the SFO kinetic principle is applicable to inhibitory ChRs, creating bistable and extremely light-sensitive inhibitory ChRs, SwiChR and SwiChR++ with excitation wavelength (on by 475nm and off by 600 nm). In addition to stability, another characteristic of step-function variants is the capacity to transform rapidly to the closed state when red-shifted light is applied, and SwiChR++ channel closure has been accelerated by applying 632 nm of light (Berndt et al., 2011, 2016). Another characteristic of step-function variants is improved light sensitivity of cells that efficiently become photon integrators for long light pulses. In fact, SwiChR++ expressing cells showed a 25-fold rise in light sensitivity relative to iC1C2 and a 200-fold boost relative to the pump-based NpHR inhibitor (Berndt et al., 2014; Yizhar et al., 2011a). To the best of our knowledge, theoretical studies of these bistable opsins have not been reported as yet. It is proposed to develop an accurate theoretical model of their photocycle and the bistable control of neural spiking in these SFO opsin-expressing neurons.

The natural anion channelrhodopsins (nACRs) from cryptophyte *alga Guillardia theta*, named GtACR1 and GtACR2, have near-perfect anion selectivity and generate several folds of larger photocurrents in mammalian cells than any earlier discussed channelrhodopsin anion, due to greater

single-channel conductivity than that of the known channel conducting ChRs (Wiegert et al., 2017). GtACR1, with its absorption peak at 515 nm, has an advantage over GtACR2, with maximal efficiency at 470 nm (Sineshchekov et al., 2015). Kim et al., have shown the crystal structure of the natural anion-conducting channelrhodopsin GtACR1 and have provided a framework for designing the next generation of optogenetic tools (Kim et al., 2018).

Malyshev et al. have recently shown that GtACR2 in reaction to light can generate both the cessation of stimuli and the generation of action potential for in cortical neurons (Malyshev et al., 2017). This research has opened up a very interesting option to use GtACR2 in the same experiment as both a cell silencer and a cell activator by varying the light stimulation pattern. ZipACR is particularly promising for inhibitory optogenetics due to its combination of larger current amplitudes than those of previously reported ACRs. The ZipACR with activation wavelength 520 nm, expressed in hippocampal neuron cultured mouse, allowed accurate photoinhibition of individual spikes in trains up to 50 Hz (Wietek et al., 2017).

To the best of our knowledge, there is no theoretical model of the photoresponse of these nACRs reported till date. It is proposed to develop accurate theoretical models of the photoresponse of these nACRs and nACR-expressing neurons. Further, the photostimulation conditions of these opsins are proposed to be optimized (Govorunova et al., 2015; Sineshchekov et al., 2016; Wietek et al., 2016).

In order to optimize the stimulation protocol, two-photon illumination provides better spatial specificity and deep penetration in scattering tissue. Recently, Forli et al., demonstrated the two-photon holographic illumination of anion conducting channelrhodopsin, namely GtACR2 with large photocurrent and high light sensitivity. Apart from this, they have shown that prolonged illumination of GtACR2-expressing neurons decreased the firing rate, and it hyperpolarized the membrane potential of the illuminated cell for the duration of the light stimulus. It is proposed to develop accurate two-photon stimulation model for the photoresponse of GtACR2 and also optimize its performance.

Cardiac optogenetics is an exciting new field in allowing low-energy to cause transmembrane current, offering a means for electrophysiological control (Entcheva, 2013). The use of ACR in neonatal rat ventricular cardiomyocytes, at light intensities four orders of magnitude lower than those needed for Arch3, reliably suppressed spontaneous beating of monolayers (Govorunova et al., 2015). The light intensities used (10 to 100 $\mu W/mm^2$) were orders of magnitude lower than what was required to produce similar behavior with light-sensitive pumps, suggesting that organ-scale excitation suppression may be feasible with GtACR in larger hearts. Most importantly, ACR expression enabled accurately controlled shortening of the possible duration of the action by switching on the light during its repolarization phase, which was not possible with previously used optogenetic instruments (Govorunova et al., 2016). Recently, Kopton et al., have demonstrated the electro-physiological and optical activation properties of GtACR1 in HEK 293T cells and found that ACR mediate depolarizing photocurrents in resting cardiomyocytes, which can be used for both activation and inhibition of cardiac activity (Kopton et al., 2018).

So far, the theoretical modelling of ACR mediated optogenetic control of cardiac cell has also not been reported. Hence, it is also proposed to formulate a theoretical model for GtACR1 mediated optogenetic control of cardiac cells.

The specific objectives of the proposed thesis are as follows:

1) It is proposed to develop accurate theoretical models for the photoresponse of the following step-function opsins:
    a) ChR2- C128A (excitatory)
    b) SwiChR++ (Inhibitory)

2) Further efforts will be made to investigate and to optimize the effect of the following factors on optogenetic mediated neural spiking in step function opsin (SFO)-expressing neurons,
    a) Intensity
    b) Pulse-width

c) Frequency

d) Wavelength

e) Expression density

3) It is proposed to formulate accurate theoretical models of the photoresponse of the following anion conducting channelrhodopsins:

a) GtACR1

b) GtACR2

4) Further efforts will be made to investigate and to optimize the effect of following factors on optogenetic control in ACR-expressing neurons for low-power and high frequency operation:

a) Intensity

b) Pulse-width

c) Frequency

d) Expression density

e) Ion-concentration

5) It is proposed to formulate an accurate computational model of two-photon optogenetic control of GtACR2-expressing neurons.

6) It is proposed to formulate a theoretical model for GtACR1 mediated optogenetic control of cardiac cells.

7) Further efforts will be made to compare the photoresponse of the above variants, from same or different classes, to develop an understanding of their use for biomedical applications.

5. Methodology

A detailed study of the photoresponse of SFOs will be undertaken to formulate accurate model of their photocycle based on experimental results reported by Berndt et al., (2009; 2015). Further, these models will be integrated with neuron circuit models to simulate optogenetic neural spiking. Theoretical simulation will be compared with recently reported experimental results.

The photocurrents in excitatory SFOs-expressed in hippocampal neurons have been recorded in response to pulses of 450 nm blue light, at -50 mV holding potential and 240 mW/cm^2 intensity. The peak photocurrent recorded in C128A has been shown at smaller photocurrent amplitude 74 ± 17 pA. The bi-stable switching behaviour has been shown in the hippocampal neurons-expressing C128A. The current clamp recording has been reported from a hippocampal neuron expressing of C128A with pairs of 470-nm and 535-nm light stimuli 10ms and 50ms respectively (Berndt et al., 2009). An effort will be made to analyze these results at low intensity.

A detailed theoretical analysis will be carried out to optimize the photoresponse with respect to important factors that include, light intensity, pulse-width, wavelength and expression density to get optimal control over neural spiking. Further efforts will be made to achieve action potential excitation and inhibition, at low power and optimized chloride gradients, in case of inhibitory SFOs.

Accurate computational models of the photocycle of GtACR1 and GtACR2 will be formulated and integrated in neurons to simulate optogenetic control of neural spiking, based on the reported experimental data (Govorunova et al., 2015; Sineshchekov et al., 2016; Wietek et al., 2016).

The photoresponse of GtACR1 and GtACR2–expressed in hippocampal neurons have been demonstrated at light intensity 3.40 mW/mm^2 (at wavelength 520 nm) and 3.12 mW/mm^2 (at wavelength 470 nm), respectively with low (10 mM) and high (150 mM) extracellular chloride concentrations. At these parameters, the holding potential has been varied from -80 mV to +40 mV, in steps of 20 mV. The GtACR2 allowed precisely controlled optical silencing of spikes at

frequencies up to 25 Hz. In GtACR2-expressing neurons full suppression of spiking was observed at 0.005 mW/mm^2 (Govorunova et al., 2015; Sineshchekov et al., 2016; Wietek et al., 2016).

In the present study, conditions for greater control over neural spiking will be evaluated in terms of intensity, wavelength, frequency, pulse-width, ion-concentration and expression density. Further, efforts will be made to optimize the parameters for low power and high frequency operations.

An accurate two-photon stimulation model of the photocycle of GtACR2 will be formulated, based on experimentally reported data (Forli et al., 2018). Further, the model will be incorporated in neuron circuit model to simulate optogenetic spiking control.

Forli et al., have found that holographic illumination of GtACR2-expressing neurons (excitation wavelength: 920 nm; stimulus power: 30 mW; stimulus duration: 500 ms) triggered clear outward currents (holding potential: -50 mV; chloride equilibrium potential: -68 mV).

Further, we will validate our model by comparing with reported experimental results. After that optimal conditions for greater optogenetic control over neural spiking will be evaluated.

A detailed theoretical study of GtACR1-mediated optogenetic control of cardiac cells, experimentally reported by Govorunova et al., (2016) and Kopton et al., (2018), will be undertaken. An accurate computational model for cardiac optogenetic stimulation using GtACR1, by incorporating the photocycle model of GtACR1 into cardiac cell circuit model, will be formulated.

It has been shown that action-potential generation can be suppressed immediately after switching on the light upon lentiviral expression of Arch in cardiomyocytes. It resumed at irregular intervals during the 30s illumination period even at the highest light intensity used 6.7 mW/mm^{-2} (Govorunova et al., 2016). Recently, Kopton et al., performed experimental demonstration in transiently transfected HEK 293T cells to characterize the GtACR1-mediated photocurrents and its ion dependence. Upon 575 nm-light applications, GtACR1 activation results in large photocurrents whose direction and amplitude depends on the Cl^- concentration gradient with light intensity of 4 mW/mm^2 (Kopton et al., 2018).

Further, we will validate our model by comparing it with reported experimental results. After that optimal conditions for greater optogenetic control over cardiac response will be evaluated.

6. Work done till Now

An extensive literature survey has been carried out in the field of optogenetics. The computational model of the photocycle of step-function opsins (SFOs), namely, ChR2(C128A) has been developed. The 3-state transition rate model accurately simulates the experimental results, of Berndt et al., (2009) which highlights the accuracy of the model.

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